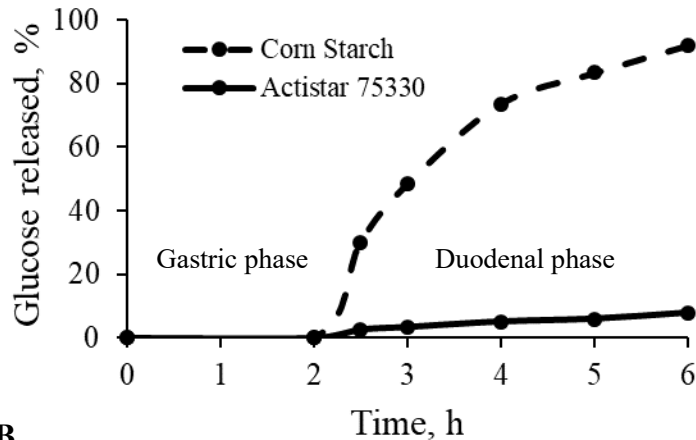
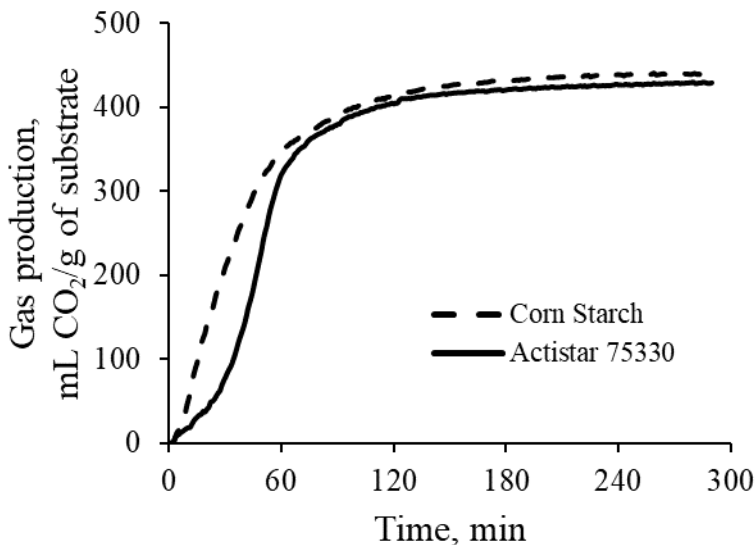


5 **A**



6 **B**



7 **Supplemental Figure S1.** In vitro evaluation of the resistant starch used during challenge periods: A.
8 Degradation of corn starch (dashed line) and resistant starch (Actistar 75330; solid line) expressed as total
9 glucose released overtime, which indicates potential enzymatic degradation in small intestine determined
10 using a method described by Garcia-Campayo et al. (2018). Approximately 90% of glucose from the corn
11 starch was released at 6 h of incubation, indicating high expected digestibility in small intestine. In contrast,
12 less than 10% of glucose from the resistant starch was released over 6 h of incubation, indicating low
13 expected digestibility; B. Fermentation of corn starch (dashed line) and resistant starch (Actistar 75330;
14 solid line) using the ANKOM RF gas production system (Macedon, NY) and rumen fluid as inoculum over
15 time, which indicates potential for hindgut fermentation when abomasally infused. Corn starch increased
16 gas production more quickly compared with resistant starch during the first 60 min, and starch sources were
17 likely not different after 60 min of incubation.
18

Supplemental Table S1. Carryover assessment that evaluates the difference between Model A (no carryover effects added) and Model B (with carryover effects). A Chi-square *P*-value < 0.05 indicates models are significantly different, and therefore, carryover effects were present.

Variables	-2 Res Log Likelihood			Chi-square
	Model A	Model B	Difference	Square test <i>P</i> -value
<i>Performance parameters</i>				
DMI, kg/d	160	157	3.58	0.17
Yields, kg/d				
Milk	240	234	6.56	0.04
Milk fat	4.66	10.8	6.14	0.05
Milk protein	-32.9	-25.6	7.30	0.03
Milk lactose	6.41	11.9	5.52	0.06
3.5% FCM	247	241	6.66	0.04
Milk variables				
Fat, %	53.8	52.8	0.981	0.61
Protein, %	-69.9	-61.6	8.37	0.02
Lactose, %	-62.8	-52.0	10.8	<0.01
SCC, x 1,000/mL	11.9	17.7	5.79	0.06
MUN, mg/dL	155	153	2.14	0.34
3.5% FCM/DMI	27.7	32.9	5.18	0.08
BW, ¹ kg	107	96.0	10.9	<0.01
BW change, ² kg	92.2	80.0	12.3	<0.01
BCS ³	18.3	23.6	5.33	0.07
BCS change ⁴	13.3	17.1	3.77	0.15
<i>Fecal parameters</i>				
Fecal pH				
4 h after morning feeding	7.93	9.70	1.77	0.41
8 h after morning feeding	-2.50	4.29	6.79	0.03
8 – 4 h after morning feeding	12.1	14.1	2.06	0.36
Fecal starch				
4 h after morning feeding	51.9	44.9	6.94	0.03
8 h after morning feeding	65.1	59.6	5.48	0.06
8 – 4 h after morning feeding	65.9	60.8	5.11	0.08
<i>Inflammatory markers⁵</i>				
LBP, µg/mL	-47.9	-40.7	7.15	0.03
Endotoxin, pg/ml	53.9	50.1	3.74	0.15
Haptoglobin, µg/mL	82.7	83.8	1.06	0.59
Serum amyloid A, µg/mL	38.8	42.5	3.73	0.16
IL1β, pg/mL	101	91.2	10.2	<0.01
<i>Metabolic parameters</i>				
NEFA, µEq/L	21.2	25.0	3.73	0.15
BHB, mg/dL	-52.4	-48.3	4.09	0.13
Glucose, mg/dL	-113	-102	10.2	<0.01

¹Measured on d 14 of the wash-out period.

²Change measured between d 5 of the challenge and 14 of wash-out period.

³Measured on d 5 of the challenge period.

⁴Change in a 21-d period.

⁵Abbreviations: LBP (LPS binding protein), IL1 β (interleukin 1 beta), NEFA = nonesterified fatty acids, BHB = beta-hydroxybutyrate, NA = not applicable.

Supplemental Table S2. Performance, fecal, metabolic, hepatic, and inflammatory parameters of cows subjected to different treatments during challenge periods. Results were adjusted for carry-over effects.

Variables ²	Treatment ¹			SEM	P-values	
	CTR	FR	RS		Trt	Trt ×Day
<i>Performance parameters</i>						
DMI, kg/d	24.1 ^a	12.4 ^b	22.3 ^a	0.9	<0.01	0.59
Yields, kg/d						
Milk	32.4	26.0	30.3	2.0	0.11	<0.01
Milk fat	1.12	1.01	1.14	0.08	0.45	0.33
Milk protein	0.98	0.79	0.93	0.06	0.09	0.01
Milk lactose	1.56	1.24	1.47	0.10	0.09	<0.01
3.5% FCM	32.2	27.7	31.6	2.1	0.24	0.13
Milk composition						
Fat, %	3.46 ^b	3.92 ^a	3.76 ^{ab}	0.11	0.05	0.39
Protein, %	3.09	3.07	3.11	0.04	0.67	0.06
Lactose, %	4.82	4.73	4.81	0.04	0.14	0.58
SCC, ³ ×1,000/mL	72	116	75		0.19	0.15
MUN, mg/dL	14.6	14.0	13.8	0.5	0.44	0.28
3.5% FCM/DMI	1.32 ^b	2.28 ^a	1.40 ^b	0.08	<0.01	0.04
BW, ⁴ kg	704	703	711	20	0.54	NA
BW change, ⁵ kg	7.5 ^a	-59.5 ^b	-2.71 ^a	5.2	<0.01	NA
BCS ⁶	2.86	2.71	2.88	0.30	0.49	NA
BCS change ⁷	0.136	-0.085	-0.032	0.155	0.56	NA
<i>Fecal parameters</i>						
Fecal pH						
4 h after morning feeding	6.84	6.81	6.58	0.13	0.42	NA
8 h after morning feeding	6.92 ^a	6.68 ^a	6.27 ^b	0.08	<0.01	NA
8 – 4 h after morning feeding	0.091	-0.161	-0.259	0.156	0.34	NA
Fecal starch						
4 h after morning feeding	2.77	1.08	2.35	0.73	0.28	NA
8 h after morning feeding	2.96	2.45	6.88	1.75	0.23	NA
8 – 4 h after morning feeding	0.18	1.37	4.67	1.82	0.25	NA
<i>Inflammatory markers⁸</i>						
LBP, ³ µg/mL	3.03	3.02	3.75		0.18	0.03
Endotoxin, ³ pg/ml	402	362	411		0.36	NA
Haptoglobin, ³ µg/mL	0.66	0.65	1.54		0.30	0.05
Serum amyloid A, ³ µg/mL	29.7	30.5	36.3		0.93	0.31
IL1β, ³ pg/mL	1,096	1,404	1,338		0.81	NA
<i>Metabolic parameters</i>						
NEFA, ³ µEq/L	75 ^c	162 ^a	97 ^b		<0.01	0.41
BHB, mg/dL	11.4 ^a	7.9 ^b	11.1 ^a	0.6	<0.01	0.45
Glucose, mg/dL	62.6	62.6	61.3	3.4	0.85	0.99

¹Treatments (Trt) were: control (CTR; fed ad libitum), 50% feed restriction (FR), and TMR fed ad libitum plus 500 g DM resistant starch (RS; abomasally infused as a pulse-dose once daily 30 min before am feeding).

²Different letters within a row indicate $P \leq 0.05$.

³Data were transformed for analysis and LSM were back-transformed and included in the table for interpretational purposes. Transformed values and SEM were not included for simplicity purposes, and *P*-value are those of the transformed data.

⁴Measured on d 14 of the wash-out period.

⁵Change measured between d 5 of the challenge and 14 of wash-out period.

⁶Measured on d 5 of the challenge period.

⁷Change in a 21-d period.

⁸Abbreviations: LBP (LPS binding protein), IL1 β (interleukin 1 beta), NEFA = nonesterified fatty acids, BHB = beta-hydroxybutyrate, NA = not applicable.